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Trichomes diversity in the tropical flora of Pakistan

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Trichomes diversity from the study of foliar epidermal anatomy has been studied for 51 species belonging to 40 genera and 20 families. In the studied plant species, 10 different types of trichomes were observed for both glandular and eglandular types. Among these species, 38 are amphitrichomic while the remaining 13 are either epitrichomic or hypotrichomic. The most frequent types of trichomes were attenuate and uniseriate types.

Key words: Trichomes, foliar epidermis, glandular, eglandular.

INTRODUCTION

The term trichomes are applied to epidermal outgrowth of diverse forms, structures and functions (Essau, 1898). Trichomes are formed on all parts of the plants including stamens (for example, *Tradescantia*) and seed (for example *Gossypium*) (Cutter, 1977). The presence of a particular type of trichomes can frequently delimit species, genera or family in taxonomic studies (Methalfe and Chalk, 1950). The adaptive values of trichomes and their possible role in plants' delimitations are areas of investigations that have just began to be utilized by the systematists, evolutionists and ecologists. The description about the morphology of the individual trichomes is not easy. The structure and nature of trichomes are somehow given great importance in phylogeny. According to Netolitzky (1932) and Carlquist (1961) Papilla, unicellular trichomes and radially symmetrical trichomes are parallel to the leaf surface which are considered to be more primitive, while those complicated ones are considered as advanced. They alter the boundary layer over leaf surface, function in light piping; alter heat loss and aid in reducing water loss through transpiration. They can also protect against herbivory, pathogens and act in storage and secretion of secondary metabolites (Agren and Schemske, 1994). Although

morphology of trichomes varies considerably, there are two major classes of trichomes; the glandular and non-glandular or epiglandular trichomes (Sinha et al., 2001). Glandular trichomes have received considerable attention in view of their capacity to synthesize, store and secrete secondary metabolites that help to protect plants against insect predation and other biotic challenges (Wagner, 1991; Ranger and Hower, 2001; Wagner et al., 2004) for example, the peltate glandular trichomes of mint produce a suite of defensive monoterpenes which are their major components and give the characteristic smell and flavor to mint oil (McCaskill et al., 1992; Voirin and Bayet, 1996). The taxonomic significance of epidermal morphology is well documented in botanical literature (Dehgan, 1980). Some particular group of plants or taxa seem to be characterized by specific type of epidermal features, which are the; epidermis, stomata, gland and trichomes (Park, 1994; Hong and Oh, Hong and Son, 1999, 2000).

Trichomes can be used as important taxonomic tools. These have also been used as an evidence to detect hybridization among different species. Redford (1974) dealt with leaf anatomy, the characters which has proven to be of systematic value are: cuticular characters, epidermis, stomata, subsidiary cells and trichomes.

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According to Krak and Patrik (2008), trichomes characters have been shown to be an important tool in taxa delimitation in many plant families. Ghahreman et al. (1999) conducted leaf epidermal studies in the genus *Hyoscyamus* L. in Iran and concluded that the most useful anatomical characters are stomatal occurrence, stomatal index, pattern of anticlinal walls and type of trichomes.

Classification of the trichomes of angiosperms has been attempted by several workers such as Weiss (1867), Bary (1884) and Cowen (1950), which resulted in systems that are artificial, serving to delimit the trichomes into various arbitrary groups. The characters which have been proven to be of systematic value are cuticular characters, epidermis, stomata, subsidiary cells and trichomes (Ellis, 1976). The present study deals with the diversity of trichomes for taxonomic purposes in some plant species from Pakistan.

MATERIALS AND METHODS

Fresh specimens were used for anatomical studies. For leaf epidermal anatomy, Shultz methods of maceration with improved techniques was followed (Subrahmanyam, 1996). For peeling of epidermis, fresh leaves were taken in a test tube covered with 4 ml of concentrated nitric acid, to which 0.2 g of potassium chloride and 1 ml of distilled water was added then the mixture was carefully boiled, after a few second the epidermis of leaves were separated in the form of thin pellicle, the contents were emptied into a Petri dish that was partly filled with water. For mounting of epidermal strips on glass slide, methods of Bokhari (1971) and Cotton (1974) were used, followed by Clark (1960). In this procedure, peeled epidermis was kept in KOH solution for 24 h. KOH solution was prepared by adding 4 to 6 KOH pellets in 30 ml of water. After 24 h, epidermal strips were washed and placed in bleach for 30 s. In the next step the epidermal strip was washed and placed in the center of glass slide with the help of forces and needles. Now 1 to 2 drops of lactic acid were poured on the epidermis. Cover slip was placed with extreme care to avoid air bubbles.

RESULTS

Keeping in view the importance of foliar epidermal trichomes, a total of 51 species belonging to 40 genera and 20 families was investigated. Ten different types of trichomes were observed, and the most frequent one is attenuate, while uniseriate types with 17.65% were found in 9 species. 38 species out of 51 species have trichomes of amphitrichomic nature in which 28 species having trichomes of same type on both sides, while the remaining 10 species are seen with different types of trichomes on abaxial and adaxial surfaces. In the remaining 13 species, 10 species are hypotrichomic while the other 3 species are epitrichomic. In the studied 20 families, eight species belongs to family Asteraceae, five from Brassicaceae, and four species each to Convolvulaceae, Amaranthaceae and Lamiaceae. The details are given in Table 1 and Plate 1. Asteraceae with

eight species of *Artemisia vulgaris* L., *Bidens biternata* Lour, *Calendula arvensis* L., *Conyza canadensis* (L.) Cronq., *Datura stramonium*, *Parthenium hysterophorus* L., *Taraxicum officinalis* and *Xanthium strumarium* L. with same trichomes on both sides in only four species as Clavate in *B. biternata* Lour, Capitata in *D. stramonium* and uniseriate in *X. strumarium* and *C. canadensis* (L.) Cronq. *P. hysterophorus* L. with uniseriate trichomes are only on adaxial surface. The remaining species has different types of trichome on both sides. *C. arvensis* L. has capitata on both sides, while it attenuates on abaxial surface. *T. officinalis* with limaciform type is found on abaxial surface and capitata on adaxial surface. The average length of their trichomes is 289.6 μm and average width is 55.50 μm , they are mostly multicellular as shown in Table 1.

Five species have been investigated for Brassicaceae as *Capsella bursa-pastoris* (L.) Medik., *Cardaria draba* (L.) Desv. Journ, *Malcolmia africana* (L.) R. Br, *Neslia apiculata* Fisch., C.A. Mey. and *Sisymbrium irio* L. They are having antler and pedate type of trichomes on both surfaces except that of *C. draba* (L.) Desv, are having attenuate type on abaxial surface while antler and pedate type on adaxial surface. The attenuate with a single cell as that of *N. apiculata* Fisch and C.A. Mey., while the antler and pedate type ranges from single cell to five cells as in *C. draba* (L.) Desv. and *M. africana* (L.) R. Br. Their average trichome length is 239.16 μm and width is 15.20 μm .

Similarly, four species of *Convolvulus arvensis* L., *Ipomoea cairica* (L.) Sweet, *Ipomoea nil* (L.) *Ipomoea purpurea* (L.) Roth have been studied, and they resulted to *C. arvensis* L., and *I. cairica* (L.) Sweet, which have a single type of trichome on adaxial and abaxial sides. The first is with attenuate type and the other one is with peltate type, the remaining two species *I. nil* (L.) Roth, have uniseriate type on abaxial surface while the peltate type is on adaxial surface. The average length of their trichomes is 231.73 μm and width is 68 μm .

Lamium amplexicaule L, *Mazus japonicus*, *Mentha longifolia* L and *Salvia aegyptiaca* studied from Lamiaceae in which *M. longifolia* L and *S. aegyptiaca* are having attenuate and peltate type of trichomes respectively on both sides while *L. amplexicaule* L are having attenuate on abaxial surface and *M. japonicus* with peltate one on abaxial side while uniseriate on adaxial surface. Their average length of trichomes is 206.18 μm and width is 57.20 μm (Table 2).

For Amaranthaceae, four species have been selected as *Achyranthes aspera* L., *Amaranthus spinosus* L., *Amaranthus viridis* L., and *Digera muricata* (L.) Mart., in which *A. aspera* L., and *A. viridis* L., are having attenuate and Clavate types of trichomes on both sides respectively. *D. muricata* (L.) Mart. with uniseriate type on abaxial surface while *A. spinosus* L., with attenuate one on abaxial side and uniseriate on adaxial side. The average length of trichomes is 198.66 μm and width is

Table 1. Details of trichomes, their qualitative and quantitative characters. NOC stands for number of cells.

Name	Family	View	Type	NOC	Size (L×W) μm
<i>Achyranthes aspera</i> L.	Amaranthaceae	Same	Attenuate	1	298×29.8
<i>Amaranthus spinosus</i> L.	Amaranthaceae	Ab	Attenuate	1	372.5×14.9
		Ad	Uniserriate	Multicellular	223.5×39.73
<i>Amaranthus viridis</i> L.	Amaranthaceae	Same	Clavate	4	149×14.9
<i>Digera muricata</i> (L.) Mart.	Amaranthaceae	Ab	Uniserriate	Multicellular	149×9.50
<i>Artemisia vulgaris</i> L.	Asteraceae	Same	Capitate	3	96.85×41
			Peltate	1	64.5×47.2
<i>Bidens biternata</i> Lour	Asteraceae	Same	Clavate	9	521.5×37.25
<i>Calendula arvensis</i> L.	Asteraceae	Same	Capitate	6 to 11	335.25×79.50
		Ab	Attenuate	1	298×34.83
<i>Conyza canadensis</i> (L.) Cronq.	Asteraceae	Same	Uniserriate	Multicellular	223.5×11.20
<i>Datura stramonium</i>	Asteraceae	Same	Capitate	Multicellular	521.5×84.43
<i>Parthenium hysterophorus</i> L.	Asteraceae	On Ad	Uniserriate	Multicellular	298×17.80
		Ab	Lamaciform	1	193.7×37.25
<i>Taraxicum officinalis</i>	Asteraceae	Ad	Capitate	3	223.5×89.5
<i>Xanthium strumarium</i> L.	Asteraceae	Same	Uniserriate	Multicellular	409.80×130.4
<i>Cynoglossum glochidiatum</i> Wall	Boraginaceae	On Ab	Attenuate	1	372.5×14.9
<i>Cynoglossum lanceolatum</i> Forssk.	Boraginaceae	Same	Attenuate	1	298×22.35
<i>Capsella bursa-pastoris</i> (L.) Medik.	Brasicaceae	Same	Antler and pedate	1	223.35×22.5
		Ab	Attenuate	1	186.25×10
<i>Cardaria draba</i> (L.) Desv., Journ	Brasicaceae	Ad	antler and pedate	5	335.25×11.20
<i>Malcolmia africana</i> (Linn.) R. Br	Brasicaceae	Same	antler and pedate	5	208.6×14.9
<i>Neslia apiculata</i> Fisch., C.A. Mey.	Brasicaceae	Same	antler and pedate	1	260.75×17.75
<i>Sisymbrium irio</i> L.	Brasicaceae	On Ab	Stellate	1	223.5×14.9
<i>Cannabis sativa</i> L.	Cannabaceae	On Ab	Attenuate	1	234.45×42.90
		Ab	Attenuate	1	238.4×22.35
<i>Silene indica</i> Roxb.	Caryophyllaceae	Ad	Peltate	1	74.5×37.5
<i>Stellaria media</i> (L.) Vill	Caryophyllaceae	Same	Peltate	1	186.25×14.9
<i>Convolvulus arvensis</i> L.	Convolvulaceae	Same	Attenuate	1	372.5×37.25
<i>Ipomoea cairica</i> (L.) Sweet.	Convolvulaceae	Same	Peltate	1	80.05×66.25
<i>Ipomoea nil</i> (L.) Roth.	Convolvulaceae	Same	Attenuate	1	301×48.55
			Peltate	1	141.55×109.22
<i>Ipomoea purpurea</i> (L.) Roth	Convolvulaceae	Ab	Uniserriate	Multicellular	3576×44.5
		Ad	Peltate	1	134.1×102.25
<i>Chrozophora sabulosa</i> Karelin & Kirilov, Bull.	Euphorbiaceae	On Ab	Stellate	1	335.25×22.35
<i>Euphorbia herta</i>	Euphorbiaceae	Same	Uniserriate	Multicellular	135.63×25.20
<i>Euphorbia indica</i> Lamarck.	Euphorbiaceae	Same	Uniserriate	Multicellular	407.75×9.16
<i>Lamium amplexicaule</i> L	Lamiaceae	Ab	Attenuate	1	223.5×24.83
		Ab	Peltate	1	89×82
<i>Mazus japonicus</i>	Lamiaceae	Ad	Uniserriate	Multicellular	238.4×47.18
<i>Mentha longifolia</i> L	Lamiaceae	Same	Attenuate	1	372.5×52.15

Table 1. Contd.

<i>Salvia aegyptiaca</i>	Lamiaceae	Same	Peltate	1	107.5×80.02
			Forked	1	372.5×26
<i>Malvastrum coromendelianum</i> (L.) Garcke	Malvaceae	Same	Antler and pedate	1	260.75×13.3
			Peltate	1	104.3×89.4
<i>Boerhavia procumbens</i> Banks ex Roxb.	Nyctaginaceae	Same	Capitate	7	312.9×65.06
			Peltate	6	275.65×33.52
<i>Epilobium hirsutum</i> L.	Onagraceae	Same	Attenuate	1	260.75×19.86
<i>Plantago lanceolata</i>	Plantaginaceae	Same	Uniserriate	multicellular	223.5×12.4
<i>Polygonum plebejum</i>	Polygonaceae	Same	Attenuate	1	228.5×20.2
<i>Anagallis arvensis</i> L.	Primulaceae	Ab	Peltate	1	89.4×74.5
<i>Verbascum thapsus</i> L.	Scrophulariaceae	Same	Attenuate	1	447×37.25
			Stellate	1	315×12.2
<i>Veronica arvensis</i> L.	Scrophulariaceae	Same	Uniserriate	3	171.35×8.63
<i>Veronica dydima</i> L.	Scrophulariaceae	Ab	Uniserriate	3	260.75×33.53
		Ad	Peltate	3	119.2×67.06
<i>Solanum melongena</i> L.	Solanaceae	Ab	Stellate	1	400.5×14.9
<i>Solanum nigrum</i> L.	Solanaceae	Ab	attenuate	1	447×29.8
		Ad	Peltate	1	216.05×93.13
<i>Solanum surattense</i> Burm.	Solanaceae	Ad	Stellate	1	186.25×14.9
<i>Corchorus olitorius</i> L.	Tiliaceae	Ab	Attenuate	1	335.25×42.21
		Ad	Peltate	2	126.70×57.18
<i>Corchorus trilocularis</i> L.	Tiliaceae	Same	Peltate	2	134.1×37.30
<i>Phyla nodiflora</i> L.	Verbenaceae	Ab	Peltate	1	59.6×29.8
<i>Vitex negundo</i> L.	Verbinaceae	Ad	Uniserriate	3	447×20.05
<i>Fagonia indica</i> Burm.	Zygophyllaceae	Ab	Anvil and Malpighiaceous	1	223.5×89.4
<i>Tribulus terrestris</i> L.	Zygophyllaceae	Same	Attenuate	1	521.5×14.9
			Peltate	1	149×134.1

18.14 µm. The details are given in Table 1.

Similarly, three species each for Euphorbiaceae, Scrophulariaceae and Solanaceae were studied as *Chrozophora sabulosa* Karelin and Kirilov, Bull, *Euphorbia herta* and *Euphorbia indica* Lamarck. For Euphorbiaceae, *Verbascum thapsus* L., *Veronica arvensis* L., and *Veronica dydima* L. for Scrophulariaceae and *Solanum melongena* L., *Solanum nigrum* L. and *Solanum surattense* Burm. from Solanaceae. Their study resulted in Euphorbiaceae *E. herta* and *E. indica* having uniseriate trichomes on both sides while *C. sabulosa* Karelin and Kirilov, Bull., having Stellate only on abaxial side. In Scrophulariaceae *V. arvensis* L., with uniseriate type on both sides while *V. dydima* L with uniseriate on abaxial side and peltate one on adaxial side. *V. thapsus* L., with attenuate and Stellate type on both sides. In

Solanaceae three species with a single genus varies in details as *S. melongena* L., with Stellate type on adaxial surface only, while *S. surattense* Burm., with Stellate type on adaxial surface but *S. nigrum* L. with attenuate type on abaxial side and peltate type on adaxial surface. The details are given in Table 1.

DISCUSSION

Sinha et al (2001) studied aerobiology, diversity and chemistry of some air borne plant trichomes between September 1995 and August 1996 at Magadh University campus, Bodh Gaya, India. They found thirty-three different types of trichomes using a Gravity Air sampler. Krak and Patrik (2008) investigated trichomes diversity

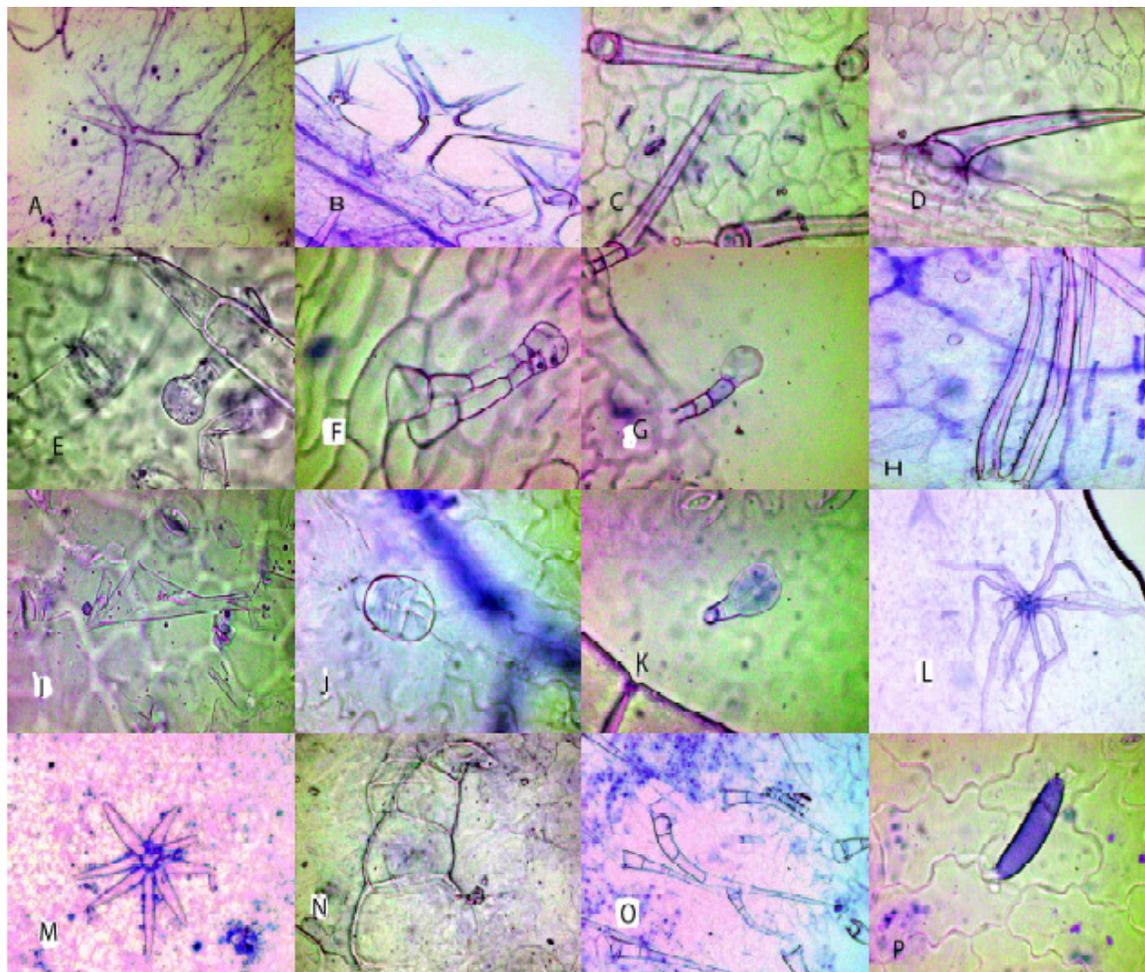


Plate 1. Showing figures of different types of trichomes as A and B antler and pedate type, C and D attenuate type, E and F capitate type, G clavate type, H Forked one, I malpighiaceus, J and K peltate type, L and M stellate type, N and O uniseriate type and P lamaciform one. Details of the species are A: *Malcolmia Africana*, B: *Neslia apiculata*, C and F: *Calendula arvensis*, D: *Ipomoea nil* E: *Datura stramonium* G: *Amaranthus viridis*, H and J: *Malvastrum coromendelianum*, I: *Fagonia indica*, K: *Veronica arvensis*, L: *Sisymbrium irio*, M: *Solanum surattense*, N: *Xanthium strumarium*, O: *Conyza Canadensis* and P: *Taraxicum officinalis*.

on stems and leaves of 135 representatives of 53 genera by light and scanning electron microscopy; trichomes are classified into main eight types and several subtypes. Aziz et al. (2004) said that “glandular trichomes are the sites of plant natural product synthesis and accumulation for protection against insect predation”. “Glandular trichomes in *Geranium* species have been shown to secrete viscous exudates that provide a defense mechanism against arthropods (Gerholo et al., 1984; Walters et al., 1989; Hesk et al., 1990; Hare and Elle, 2002). Osmophores is a form of glandular epidermis common in floral tissues, it secrete volatile compounds responsible for attracting pollinators (Lehnebach and Robertson, 2004). Trichomes from tomato species collectively produce a number of insecticidal sesquiterpenes, acyl sugars and methyl ketones (Li et al., 1999; Antonious, 2001; Maluf et al., 2001). In *Plantago ovata*

Forsk, the trichomes were unicellular and glandular and their main features were present abundantly. Epidermal structures like trichomes – their scanty distributions as well as poor development had been envisaged to be one of the major causes for high rate of transpiration leading to low rate of survival in cases of micro propagated plants (Brutti et al., 2002).

Conclusion

This study revealed the presence of variations in trichomes types at different taxonomic levels. Differences were observed at abaxial and adaxial surfaces of the same leaf and even at the same surface. So it is deduced from this work that more comprehensive research is necessary for their further elaboration.

REFERENCES

- Agren J, Schemske DW (1994). Evolution of trichome number in a naturalized population of *Brassica rapa*. *Am. Natl.* 143:1-13.
- Antonious GF (2001). Production and quantification of methyl ketones in wild tomato accessions. *J. Environ. Sci. Health B36*:835-848.
- Aziz N, EN LP, Gregory DM, Richard AD (2004). Transcriptome analysis of alfalfa glandular trichomes. *Planta*, 2005(221):28–38.
- Bary DA (1884). Comparative anatomy of the vegetative organs of the Phaenerogams, Berlin.
- Brutti CB, Rubio EJ, Llorente BE, Apostolo NM (2002). Artichoke leaf morphology and surface features in different micropropagation stages. *Biol. Plant* 45:197-204.
- Clark J (1960). Preparation of leaf epidermis for topographic study. *Stain Technol.*, 33: 35-39.
- Cowen JM (1950). The Rhododendron leaf. A study of the epidermal appendages. *Q. Rev. Biol.* 27(2):214-215.
- Dehgan B (1980). Application of epidermal morphology to taxonomic delimitations in the genus *Jatropha* L. (Euphorbiaceae). *J. Bot. J. Linn. Soc.* 80:257–278.
- Ellis RP (1976). A procedure for standardizing comparative leaf anatomy in Poaceae.1. The leaf blade as viewed in transverse section. *Bothalia* 12:65-109.
- Gerholo DL, Craig R, Mamma RO (1984). Analysis of trichome exudate from mite resistant geraniums. *J. Chem. Ecol.* 10:713-722.
- Ghahreman, AM, Khatams Az, Ganj-Karimi M (1999). Leaf epidermal studies in the genus *Hyoscyamus* L. (Solanaceae) in Iran. *Iran. J. Bot. Tehran* 8(1):81-90.
- Hare JD, Elle E (2002). Variable impact of diverse insect herbivores on dimorphic *Datura wrightii*. *Ecology*, 83: 2711–2720.
- Hesk D, Collins LC, Craig R, Mamma RO (1990). Arthropod-resistant and susceptible geraniums. Comparison of chemistry. In Hedin PA (ed) *Naturally occurring pest bioregulators*. ACS Symposium Series 449. Washington, pp. 224–250.
- Hong SP, Son SP (2000). The taxonomy consideration of leaf epidermal microstructure in the tribe Rumiceae Dum. [Polygonaceae J]. *Kor. J. Plant Tax.* 30:105-121.
- Hong SP, Oh IC (1999). The taxonomic study of leaf epidermal microstructure in the genera *Polygonum* L. s. str. And *polygonella* Michx. (Polygoneae- Polygonaceae) J]. *Kor. J. Plant Tax.* 29:75–90.
- Krak KP Mráz P (2008). Trichomes in the tribe Lactuceae (Asteraceae) – taxonomic implications. *Biologia*, 63/5: 616-630.
- Lehnebach CA, Robertson AW (2004). Pollination ecology of four epiphytic orchids of New Zealand. *Ann. Bot.* 93:773–781.
- Li AX, Eannetta N, Genghis GS, Steffens JC (1999). Glucose polyester biosynthesis. Purification and characterization of an acyltransferase. *Plant Physiol.* 121:453-460.
- Maluf WR, Campos GA, das Gracas CM (2001). Relationships between trichome types and spider mite (*Tetranychus evansi*) repellence in tomatoes with respect to foliar zingiberene contents. *Euphytica* 121:73–80.
- McCaskill D, Gershenzon J, Croteau R (1992). Morphology and monoterpene biosynthetic capabilities of secretory cell clusters isolated from glandular trichomes of peppermint (*Mentha piperita* L.). *Planta* 187:445–454.
- Park KW (1994). A taxonomy study of the Magnoliaceae [J]. *Res. Rep. For. Res. Inst.* 50:173-190.
- Ranger CM, Hower AA (2001). Glandular morphology from a perennial alfalfa clone resistant to the potato leafhopper. *Crop Sci.* 41:1427-1434.
- Redford AE (1974). *Vascular plant systematics*. Harper and Row, publishers New York, Evanston, San Francisco, London pp. 182-183.
- Sinha A, Krishn BM, Raju K (2001). Aerobiology, biodiversity and chemistry of plant trichomes in the tropics at Bodh Gaya, India – a biopollutant and the suspected human allergen. *Aerobiologia* 17:261-267.
- Voirin B, Bayet C (1996). Developmental changes in the monoterpene composition of *Mentha X piperita* leaves from individual peltate trichomes. *Phytochemistry* 43:573–580.
- Wagner GJ (1991). Secreting glandular trichomes: more than just hairs. *Plant Physiol.* 96:675–679.
- Wagner GJ, Wang E, Shepherd RW (2004). new approaches for studying and exploiting an old protuberance, the plant trichome. *Ann. Bot.* 93:3-11.
- Walters DS, Grossman HH, Craig R, Mamma RO (1989). Geranium defensive agents IV. Chemical and morphological basis of resistance. *J. Chem. Ecol.* 15:357–372.
- Weiss A (1867). 'The trichomes, Karsten *Bot. Unters* 1:369-677.